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EFFECTS OF MODIFIED ATMOSPHERE PACKAGING ON SHELF-LIFE OF INDIAN MACKEREL (*RASTRELLIGER KANAGURTA*) DURING CHILLED STORAGE

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ABSTRACT

The present study was carried out, to evaluate shelf life and the effects of vacuum packaging, sodium acetate dip treatment and modified atmosphere (MA) packaging on biochemical, microbiological and sensory characteristics of gutted Indian mackerel (Rastrelliger kanagurta), during chilled storage. The gutted whole fish were dip treated in 2% sodium acetate (SA), followed by vacuum packaging in polyester laminated polyethylene bags. MA packaging of fish, was carried using high impact polystyrene (HIPS) trays in atmosphere of 60:40 carbon dioxide and nitrogen gas mixture. The packaged fish were stored under ice in 2:1 ratio of ice to fish samples in insulated boxes. The changes in total volatile base nitrogen, tri methylamine nitrogen, free fatty acid content, alpha amino nitrogen, thiobarbituric acid value, total plate count of aerobic bacteria and sensory characteristics were evaluated on every three days for a period of 33 days. Based on the biochemical and sensory indices, the untreated air packed samples were acceptable up to 9 days. The untreated vacuum packed and treated air-packed samples were acceptable up to 21and 24 days respectively; whereas SA treated vacuum packed sample was acceptable up to 27 days. The fresh whole mackerel packed in HIPS trays under air and MA packaged samples had a shelf life of 12 and 30 days respectively during chilled storage.

KEYWORDS: Mackerel, Chilled Storage, Modified Atmosphere, Vacuum Packaging & Shelf Life

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Original Article

INTRODUCTION

The present fish utilization trends in India indicate that the major quantity of fish landed at fishing harbours is marketed in fresh iced conditions, i.e. about 70% of total marine fish production. Fresh fish are highly perishable as they contain high water activity, a great load of bacteria on the gills and in gut that represents the environment from which fish are caught and the oxidation of lipids by molecular oxygen are some of the important processes that decay the fish upon harvest. There are several methods to preserve fresh fish but icing is the most common method followed for short-term preservation.

Fish packaging for retail marketing is considered an essential step in ensuring food safety and security. This will also strengthen the preservation of good quality fish for longer duration. Presently, fresh fish stored with ice has a limited shelf life of 3 to 5 days; which needs to be extended to ensure supply of fresh fish in remote areas and to increase their availability and consumption for larger number of consumers. Vacuum packaging involves the removal of air from the package, then application of hermetic seal. Vacuum packaging can considerably extend the

viable shelf life of many foods (Dalgaard et al., 1993; Srinivasa Gopal et al., 1999). Several workers have reported that, vacuum packaging in combination with chemical preservatives, such as sodium acetate will enhance the shelf life of fish in ice, considerably (Brewer et al., 1992; Kim et al., 1995).

Modified atmosphere packaging (MAP) methods have recently become more common, because of consumer demands for fresh foods. It is a promising technique, for preservation of seafood as it gives better shelf life extension, compared to mere icing of fish (Yesudhason et al., 2009). The use of MAP to store food products can increase shelf life significantly, which reduces economic loss, allows for longer distribution distances and supplies a better quality product (Farber et al., 1990). MAP, utilizing combinations of carbon dioxide, nitrogen and oxygen has become common in many countries for both storage and retail display of meat / fish. However, limited information is available on the preservation / packaging of fresh Indian mackerel despite the commercial importance of this fish. The main objective of the study was to determine the effects of vacuum and MAP on the shelf life of Indian mackerel during chilled storage.

MATERIALS AND METHODS

Fresh fish (*Rastrelliger kanagurta*) weighing 150g each, caught by purse seine fishing operations were procured from Ratnagiri fishing harbour, and brought to the laboratory in iced condition. The fishes were gutted and washed with chilled 2 ppm chlorinated water, drained well and subjected to further processing. The fishes were divided into four lots. Lot I (control air packed - CAP) comprising fish packed without vacuum, Lot II (control vacuum packed - CVP) comprising fish packed under vacuum, Lot III (treated air packed - TAP) consisted of fish dip-treated with 2% (W/V) aq. solution of sodium acetate (SA) for 30 min and packed without vacuum and Lot IV (treated vacuum packed - TVP) wherein treated fish were vacuum packed. Fish were packaged in laminated pouches made of 12 um polyester and 250 guage polyethylene, for MA packaging, fish were packed with in numbers in a HIPS tray. The trays of dimensions 185x127x45 mm, with thickness of 1600 guage were used. The trays were sanitized by dipping in 2 ppm chilled chlorine water, before use. The MAP machine model S-220MP (Vac-Star, Switzerland) was used for sealing of trays with fish and various combinations of gases, like carbon dioxide and nitrogen. Trays were sealed with a flexible packaging film of 12 um polyester laminated, with 70 um polyethylene. The ratio of volume of the gas to fish was 3:1. Food grade CO_2, and N_2 gases were used, for packaging of fish in trays. The gas composition was adjusted, by using the gas mixer of the MAP machine. Each time the control samples (air packed) of fish were also packed. The gas composition of 60:40 for CO_2+N_2 was used for packaging of mackerel. The vacuum and MAP samples of fish were stored at 0-2 ^oC, using flake ice in insulated polystyerene boxes of 70L capacity, for a period of 33 days. Re-icing was done everyday, to supplement for the melt ice. Samples were drawn on every three days, to evaluate their biochemical, microbiological and sensory characteristics. All the measurements were performed in triplicate, unless otherwise stated. The headspace gas analyser was used to analyse the composition of gases, present in the packaged trays at the time of sealing of the trays with fish, using PBI-Dansensor gas analyzer (Netherlands) model - Checkpoint O_2/CO_2. The water extractives at 49 ^oC in 24 h, from the packaging material were estimated according to USFDA 176:170 and found to have a value of 3.8 ppm.

The pH of fish meat was measured using Systronics digital pH meter. About 10 g meat was homogenised with 50 ml distilled water, and pH was measured. Total volatile base nitrogen (TVB-N) and tri methylamine nitrogen (TMA-N) were determined, using micro diffusion method (Conway, 1950). Determination of alpha amino nitrogen (AAN) was done, according to Pope and Stevens (1939), free fatty acid (FFA) content was analysed according to AOAC (2002) and thiobarbituric acid (TBA) value was determined, by the method of Tarladgis et al (1960) and total plate count of aerobic

bacteria was estimated, according to Tomlinson (1995) and enterobacteriaceae (EB) count was performed using McConkey agar medium. Sensory evaluation was done, using a panel of five trained panellists for evaluation of attributes like odour, colour, texture and overall appearance on a hedonic scale 0-9 and average values of the scores of five panellists were noted, as described by Shalini et al (2000).

RESULTS AND DISCUSSIONS

There has been a recent interest in vacuum and MA/ tray packaging of fresh fish, in order to expand market potential, to reduce waste during distribution and to ensure safety and security of fish products, to meet the demand for fish products. Indian mackerel is popular in domestic markets of India and consumed all along the maritime regions of the country. The fish keeps well in chilled conditions under air for 3-5 days, like any other fatty fishes, and are not of acceptable quality after this period. It was felt necessary to develop a suitable medium of packaging for safety and distribution of fresh mackerel in Indian retail fish markets.

The changes in the biochemical, microbiological and sensory characteristics in the chill stored vacuum packaged and MA packaged Indian mackerel samples are presented in Table 1 and 2 respectively. The initial pH of the fish was 6.3 and on storage it increased significantly ($P<0.05$) in control and treatment packs. The increase in pH may be attributed to the production of volatile basic compounds due to autolytic activites. Reddy et al (1997) reported that, increase in pH of air-packaged tilapia stored under refrigeration temperature, may be due to the production of volatile base compounds, such as ammonia by bacterial action. Similar results have been reported by Zhuong et al (1996), in shrimps treated with 2% SA and Shalini et al (2000) in *Lethrinus lentjan* fillets treated with 2% SA and vacuum packaging. However, the values of pH showed no significant (p/0.05) changes, for pearl spot packed under air and MAs during storage (Lalitha et al., 2005)

TMA is often used as an index, to assess the keeping quality and shelf life of marine fish (Hebard et al., 1982). TMA-N values of mackerel showed increasing trend in control and treated packs. The values were significantly higher in control packs in the initial 5 days as compared to SA treated and MA packaged samples. Its value reached the point of rejection on 9th day (25 – 28 mg%), in untreated samples; whereas, similar values were observed in CVP and TVP samples on 21st and 27th day, respectively and MA packed samples on 18th day. The delay in the formation of TMA may be due the lower bacterial load in the samples. The inhibitory effect of SA over the growth of bacteria may be the reason for the delayed formation of TMA-N. Dalgaard (1983) reported that, TMA-N appears to be the appropriate index, for assessing microbial spoilage of vacuum and MA packed cod fillets, stored at 0 ^{o}C and a level of 30 mg% was the upper limit of acceptability.

According to Lannelongue (1980), TVB-N comprises of mainly TMA and ammonia, that are produced by both bacterial and autolytic enzymes. The TVB-N values were found to increase, with storage period in all the experimental samples ($p<0.05$). Its value increased to 35 mg%, in CAP on 9th day of storage. Similar values were observed in CVP, TAP and TVP samples on 21st and 24th day, respectively.

Lipid hydrolysis in fish results in the formation of free fatty acids (Huss, 1971). In the present study, the FFA increased significantly ($p<0.05$) with storage time and the increase in CAP was observed to more than that in CVP and TVP samples. Husang et al (1991) observed that, vacuum and MA packaging did slow down the lipid hydrolysis, in channel catfish during storage in ice. However, concentrations of FFA may not be considered as a reliable index of spoilage in vacuum MA packed fish fillets.

The increase in levels of AAN in fish post-mortem is considered as an indication of proteolytic activities. On the day of rejection of CAP samples, the AAN value reached 69 mg%. On the contrary, the values were much lower in treated and treated-vacuum packed samples. The TBA value in the range of 1 – 3 mg malondialdehyde /kg of fish is taken as the limit of acceptability (Lakshamanan, 2000). The initial TBA value of CAP sample was 0.3 mg malondialdehyde/kg and increased significantly, to a value of 2.9 mg malondialdehyde/kg on day of rejection, i.e. 9^{th} day of chilled storage indicating that, lipid oxidation occurred. However, the TBA values on CVP, TAP and TVP samples were much lower till 12 days of storage, as compared to the CAP samples. This suggested that, vacuum packaging and also MA packaging, that limited the access of oxygen in the packs that had significantly retarded lipid oxidation of fish samples. Pastoriza et al (1996), reported increased TBA values in salmon slices and hake slices, stored under MA, compared to samples stored under air.

Microbial growth in fresh seafood is one of the major factors, associated with quality deterioration, spoilage and economic loss (Zhuong et al., 1996). The TPC was found to increase significantly with storage period. The lower values of TPC in treated and treated vacuum packed samples might be due to the preservative action of sodium acetate. This is in agreement with that of Kim and Hearnsberger (1994) and Shalini et al (2000), who reported lower count in catfish and *L. lentjan* fillets, respectively, that were treated with SA.

Fresh mackerel fish are generally considered to have very high overall acceptability. There was a significant reduction ($p<0.05$) in sensory characteristics of all samples with the storage period increased. As the days of storage in ice progressed, the sweet taste of fish muscle was lost and the texture became soft. All samples developed a fishy odour as the storage time increased. The vacuum packaged CAP samples were acceptable up to 9 days, whereas; CTP, TAP and TVP samples were acceptable to 21,24 and 27 days respectively. However, air packed samples were acceptable upto 12 days and MA packaged samples were acceptable up to 30 days.

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REFERENCES

1. *AOAC (2002). Official methods of analysis (17^{th} edn). Association of Official Analytical Chemists. Washington DC*
2. *Brewer, M.S. Mckeith, F., Martin, S.E., Dalmier, A.W. and Wu, S.Y. (1992) Some effects of sodium lactae on shelf life, sensory and physical acharacteristics of vacuum packaged beef bologna. J. Food Qual., 15:363-382*
3. *Conway, E.J. (1950) Micro- diffusion analysis and volemtric error. Crosby. Lockwood. London, UK*
4. *Dalgaard, P., Gram, L. and Huss, H.H.(1993) Spoilage and shelf life of cod fillets packed in vacuum or modified atmosphere. Int.J.Food. Microbiol., 19:283-294.*
5. *Farber, J.M., Warburton, D.W. Gour, L., Milling, M. (1990) Microbiological quality of foods packed under modified atmospheres. Food Microbiol., 7: 327-334.*
6. *Hebard, C.E.; Flick, G.J. and Martin, R.E. (1982). In Chemistry and Biochemistry of Marine Food Products (Martin, R.E., Flick, G.J. and Word, D.R. Eds) p. 149 AVI Publishing Co., Westport, CT.*

7. *Ramandeep Kaur, Ashok Kumar, BVC Mahajan & M Javed, Control of Pericarp Browning and Quality Retention of Litchi Fruit by Post-Harvest Treatments and Modified Atmosphere Packaging, International Journal of Agricultural Science and Research (IJASR), Volume 4, Issue 2, March - April 2014, pp. 38-50*
8. *Husang, D.N., Koehler, P.E., Eitenmiller, R.R. and Guilar, D.A. (1991). In Fifteenth Annual Conference on Tropical and Subtropical Fisheries. Technological Conference of the Americas i second joint meeting with Atlantic Fisheries Technology Conference, Orlando, Florida, 2-5 Dec., 1990.*
9. *Huss, H.H. (1971) In Fish Inspection and Quality Control (Kreuzer, R Ed.) p. 60 Fishing News Books Ltd, Farnham, UK.*
10. *Kim, C.R., Hearnsberger, J.O., Vickery A.P., White, V.H. and Marshall, D.I.(1995). J. Food Sci., 60: 25*
11. *Kim, C.R. and Hearnsberger, J.O. (1994). J. Food Sci., 59:513.Lakshamanan, P.T. (2000) Fish spoilage and quality assessment. In Quality assusrance in seafood processing. (TSG Iyer, MK kanoran, M Thomas and PT Mathew Eds) p. 26-40. Society of Fisheries technologists, Cochin. India*
12. *Lalitha, K.V, Sonaji, E.R., Manju, S., Jose, L., Gopal, T.K.S. and Ravishankar, C.N. (2005) Microbiological and biochemical changes in peral spot (Etroplus suratensis) stored under modified atmospheres. J. Appl. Microbiol., 99:1222-1228.*
13. *Lannelongue, M. (1980) Storage characteristics of fresh fish packed in modified atmosphere containing CO_2. Masters Thesis, Texas A & M University College Station, USA.*
14. *Pastoriza, L., Sampedro, G., Herrera, J.J. and Cabo, L.M. (1996) Effect of modified atmosphere on shelf life of iced fresh hake slices. J. Sci. Food Agric., 71:541-547*
15. *Pope, C.G. and Stevens, M.F. (1939) Biochem. J., 33:1070*
16. *Reddy, N.R., Romam, M.G., Soloman DA, Kautter, D.A. and Rhodehamel, E.J.(1997) J. Food Sci., 62: 878.*
17. *Shalini, R., Indra Jasmine, G, Shanmugam, S.A. and Ramkumar, K. (2000) Sodium acetate and vacuum packaging to improve shelf life of refrigerated Lethrinus lentjan fillets. Fish. Technol., 37(1): 8-14.*
18. *Srinivasa Gopal, T,K. Joseph, J. And Balachandran, K.K. (1999) development of fish products employing hurdle technology. In Preservation of food by hurdle technology, DFRL, Mysore. P. 95-100.*
19. *Tarladgis, B.G., Watta, B.M. and Younthan, M.T. (1960) A distillation method for the quantitative determination of malonldehyde in rancid foods. J. Am. Oil Chemists. Soc., 37:44.*
20. *Tomlinson, L.A. (1995). Bacteriological Analytical Manual. 18^{th} Edn AOAC. Washington DC.*
21. *Yesudhason, P., Gopal, T.S.K., Ravishankar, C.N., Lalitha, K.V. and Ashokkumar, K.(2009) effect of modified atmosphere packaging on chemical, textural, microbiological and sensory quality of seer fish (Scomberomorous commerson) steaks packed in thermoformed trays at 0 – 2^oC. J. Food Proc. Preser., 1745.*
22. *Zhuong, R.Y., Huang, Y.W. and Beucher, Y.Z. (1996) J. Food Sci., 61:241.*

APPENDICES

Table 1: Changes in Biochemical, TPC and Sensory Characteristics in Indian Mackerel Packed under Vacuum and Treated with 2% Sodium Acetate during Chilled Storage

Parameters	Storage Days	Fish Samples			
		CAP	TAP	CVP	TVP
pH	0	6.3±0.02	6.4±0.04	6.4±0.04	6.4±0.01
	3	6.5±0.04	6.4±0.04	6.3±0.01	6.1±0.01
	6	6.6±0.02	6.5±0.02	6.5±0.01	6.4±0.01
	9	6.8±0.04	6.5±0.04	6.6±0.01	6.3±0.02
	12	6.8±0.02	6.6±0.02	6.6±0.01	6.4±0.01
	15	ND	6.6±0.04	6.7±0.01	6.5±0.01
	18	ND	6.7±0.04	6.7±0.01	6.6±0.02
	21	ND	6.7±0.02	6.7±0.02	6.7±0.02
	24	ND	6.7±0.04	6.6±0.01	6.7±0.02
	27	ND	ND	6.5±0.01	6.5±0.04
	30	ND	ND	ND	ND
TMA-N (mg%)	0	4.92±0.12	4.92±0.05	4.92±0.04	4.92±0.8
	3	12.9±0.06	6.4±0.08	5.7±0.6	6.4±0.08
	6	13.5±0.08	8.3±0.14	7.6±1.12	8.3±0.14
	9	25.3±0.10	9.3±0.16	8.3±0.08	9.3±0.16
	12	35.1±0.20	13.6±0.20	9.8±0.08	13.6±0.20
	15	ND	14.0±0.80	12.7±1.14	14.0±0.80
	18	ND	18.3±0.06	16.3±1.1.6	18.3±0.06
	21	ND	25.2±0.08	16.3±1.1.4	26.2±0.08
	24	ND	36.6±1.2	20.1±1.1.4	25.3±1.14
	27	ND	ND	28.36±0.8	25.7±1.12
	30	ND	ND	ND	ND
TVB-N (mg%)	0	8.2±0.12	8.20±0.08	8.2±1.16	8.2±0.08
	3	10.5±0.80	8.5±1.16	8.2±1.16	8.3±0.12
	6	34.8±0.08	10.7±0.12	10.3±0.80	12.3±1.16
	9	35.9±0.80	14.6±0.80	15.4±1.16	16.3±0.80
	12	40.5±0.12	18.8±0.12	18.3±1.16	19.6±1.16
	15	ND	22.6±1.16	22.6±0.12	19.5±0.08
	18	ND	30.8±0.12	24.8±1.16	29.7±1.16
	21	ND	35.8±0.08	30.3±0.08	30.5±0.12
	24	ND	42.2±0.80	36.4±1.16	33.8±1.16
	27	ND	ND	48.6±0.12	52.2±0.80
	30	ND	ND	ND	ND
FFA (mg% Oleic acid)	0	2.9±0.02	2.9±0.04	2.9±0.04	2.9±0.01
	3	3.5±0.04	3.1±0.04	3.3±0.01	3.3±0.01
	6	3.8±0.02	3.2±0.02	3.4±0.01	3.1±0.01
	9	3.9±0.04	3.3±0.04	3.5±0.01	3.2±0.02
	12	4.2±0.02	3.5±0.02	3.9±0.01	3.4±0.01
	15	ND	3.8±0.04	4.2±0.01	3.5±0.01
	18	ND	3.7±0.04	4.3±0.01	3.6±0.02
	21	ND	4.5±0.02	3.9±0.02	3.7±0.02
	24	ND	4.0±0.04	5.1±0.01	3.9±0.02
	27	ND	ND	5.4±0.01	4.2±0.04
	30	ND	ND	ND	ND
AAN (mg%)	0	20.0±0.18	18.0±0.23	20.0±0.35	20.0±0.35
	3	28.0±0.35	22.1±0.18	22.0±0.35	26.0±0.35
	6	34.0±0.04	27.0±0.04	27.0±0.18	30.0±0.04
	9	52.0±0.35	44.2±0.35	38.5±0.04	38.0±0.18
	12	69.2±0.35	65.3±0.35	44.7±0.35	43.5±0.18
	15	ND	68.1±0.04	45.7±0.04	44.8±0.18

Table 1: Contd.,					
	18	ND	72.7±0.35	47.1±0.35	45.3±0.04
	21	ND	77.5±0.35	50.2±0.04	45.7±0.35
	24	ND	78.5±0.35	52.1±0.18	46.8±0.35
	27	ND	ND	63.1±0.18	47.3±0.04
	30	ND	ND	ND	ND
TBA (mg malondialdehyde/kg)	0	0.3±0.04	0.3±0.12	0.3±0.16	0.3±0.04
	3	1.5±0.12	0.4±0.04	0.6±0.12	0.4±0.12
	6	2.7±0.16	1.4±0.16	0.8±0.04	0.6±0.16
	9	2.9±0.16	1.9±0.12	0.8±0.16	0.8±0.04
	12	3.5±0.04	2.2±0.04	1.4±0.12	1.2±0.12
	15	ND	3.5±0.16	2.4±0.16	2.4±0.12
	18	ND	3.63±0.16	3.6±0.12	2.5±0.04
	21	ND	3.78±0.04	3.7±0.12	3.5±0.16
	24	ND	3.92±0.16	3.8±0.16	3.5±0.16
	27	ND	ND	3.8±0.16	4.2±0.14
	30	ND	ND	ND	ND
TPC (cfu/g)	0	$2.13 X10^3$	$2.13 X10^3$	$2.13 X10^3$	$2.13 X10^3$
	3	$3.51 X10^3$	$2.65 X10^3$	$2.35 X10^3$	$2.23 X10^3$
	6	$5.60 X10^3$	$4.14 X10^3$	$4.12 X10^3$	$3.61 X10^3$
	9	$6.17 X10^3$	$4.63 X10^3$	$4.59 X10^3$	$3.90 X10^3$
	12	$7.50 X10^3$	$1.90 X10^4$	$2.43 X10^4$	$4.40 X10^3$
	15	ND	$2.23 X10^4$	$3.45 X10^3$	$1.25 X10^4$
	18	ND	$3.56 X10^4$	$5.12 X10^3$	$2.50 X10^4$
	21	ND	ND	$6.20 X10^4$	$2.23 X10^5$
	24	ND	ND	$6.40 X10^4$	$2.20 X10^5$
	27	ND	ND	$6.40 X10^5$	$2.25 X10^5$
	30	ND	ND	ND	ND
Sensory score (0-9)	0	9.6±0.4	9.8±0.6	9.8±0.8	4.8±0.4
	3	9.2±0.4	9.6±0.4	9.6±0.4	9.5±0.8
	6	7.2±0.6	8.8±0.4	9.2±0.4	9.4±0.4
	9	7.0±0.6	8.6±0.6	8.2±0.8	9.0±0.4
	12	6.0±0.4	7.6±0.6	8.2±0.6	8.6±0.8
	15	6.5±0.4	7.8±0.4	7.8±0.8	8.2±0.6
	18	5.0±0.6	6.2±0.6	7.2±0.4	7.8±0.8
	21	ND	6.0±0.6	7.3±0.8	7.5±0.4
	24	ND	5.0±0.4	6.2±0.8	7.5±0.8
	27	ND	ND	6.0±0.4	6.2±0.4
	30	ND	ND	ND	ND

ND = Not done

Table 2: Changes in Biochemical, Microbiological and Sensory Characteristics in Indian Mackerel Packed under Modified Atmosphere during Chilled Storage.

Parameters	Storage Days	Samples		Parameters	Storage Days	Samples	
		CAP	MAP			CAP	MAP
pH	0	6.46±0.02	6.30±0.02	TMA-N (mg%)	0	8.24 ± 0.03	8.24±0.22
	3	6.60±0.02	6.0±0.02		3	20.22±0.12	10.20±0.18
	6	6.55±0.04	6.34±0.04		6	22.00±0.15	14.30±0.24
	9	6.60±0.04	6.50±0.04		9	28.80±0.22	18.80±0.26
	12	6.72±0.02	6.72±0.04		12	29.60±0.32	23.40±0.22
	15	6.50±0.04	6.60±0.04		15	30.40±0.25	25.40±0.14
	18	6.68±0.04	6.68±0.02		18	37.00±0.28	25.00±0.21
	21	ND	6.55±0.04		21	ND	26.20±0.42
	24	ND	6.55±0.04		24	ND	26.80±0.36
	27	ND	6.60±0.04		27	ND	30.31±0.34
	30	ND	6.75±0.04		30	ND	31.58±0.16

Table 2: Contd.,							
	33	ND	6.88±0.04		33	ND	34.68±0.35
TVB-N (mg%)	0	5.80±0.07	5.0± 0.34	APC (Log cfu/g)	0	1.0	1.0
	3	12.60±0.22	5.6±0.20		3	1.3	1.3
	6	15.0±0.21	11.2±0.32		6	2.4	1.4
	9	23.56±0.12	13.2±0.34		9	2.5	2.2
	12	29.00±0.24	15.0±0.35		12	3.4	3.1
	15	33.44±0.30	19.0±0.26		15	4.6.	4.2
	18	42.00±0.45	23.9±0.24		18	4.8	4.4
	21	ND	24.0±0.28		21	ND	4.4
	24	ND	24.2±0.08		24	ND	4.8
	27	ND	30.6±0.25		27	ND	5.3
	30	ND	42.8±0.24		30	ND	5.4
	33	ND	44.7±0.28		33	ND	5.8
EB count (Log cfu/g)	0	--	--	Sensory score (0-9)	0	9.2	9.6±0.04
	3	1.0	1.0		3	8.3±0.16	9.2±0.16
	6	1.3	1.1		6	7.4±0.04	8.9±0.12
	9	2.1	1.3		9	6.4±0.12	8.6±0.16
	12	2.3	1.8		12	6.2±0.16	8.4±0.16
	15	2.3	2.3		15	5.2±0.16	7.6±0.16
	18	2.6	2.6		18	4.8±0.12	7.6±0.04
	21	ND	2.6		21	ND	7.2±0.16
	24	ND	2.4		24	ND	7.0±0.16
	27	ND	2.8		27	ND	6.5±0.14
	30	ND	3.0		30	ND	6.5±0.16
	33	ND	3.2		33	ND	4.5±0.16

ND = Not done